28-DAY TOXICITY

OF

IN BEAGLE DOGS

SPONSOR: **Toxicology & Pharmacology Branch**
Developmental Therapeutics Program
Division of Cancer Treatment
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892

PROJECT OFFICER:

CONTRACT TYPE: Cost Plus Fixed Fee

CONTRACT NUMBER:

CONTRACTOR:

PRINCIPAL INVESTIGATOR:

STUDY DIRECTOR:

PROPOSED IN-LIFE PHASE:

Start:

Finish:

I. OBJECTIVE

The objective of this study is to determine target organ toxicity and its reversibility in dogs treated orally with for 28 consecutive days.

II. MATERIALS AND METHODS

A. Test and Control Article:

1. Name of Test Article:

2. Name of Control Article:

3. Characterization and Documentation of Methods of Synthesis, Fabrication or Derivation:

a. Test Article:

Compound identity, strength, quality, stability and purity as well as documentation of methods of synthesis, fabrication or derivation are the responsibility of the NCI. Confirmation of identity will be done immediately upon receipt of each shipment of the compound. Sufficient quantity of drug shall be reserved for archiving from each lot and shipment used.

b. Control Article:

4. Stability and Storage:

a. Test Article:

b. Control Article:

5. Formulation Preparation, Stability and Storage:

a. Test Article:

b. Control Article:

6. Dose Concentration and Homogeneity Analyses:

Dose concentration analyses will be performed on each newly formulated dosing solution prior to dosing. Homogeneity analysis will not be required, since homeogeneity has been previously demonstrated. Dosing solutions must be within ±10% of the theoretical concentration and all results of these analyses will be submitted to the NCI Project Officer within 7 days after each dosing. An adequate quantity of each dosing mixture will be retained for possible analysis until the acceptance of the final report on this compound.

B. Test System:

1. Species, Strain Supplier and Test System Justification:

Purebred beagles supplied by the DTP, DCT, NCI will be used in this study. This is an accepted species to support studies of compounds used or intended for use in humans.

2. Initial Age, Sex and Weight:

Male and female dogs will be approximately 8 to 12 months of age and approximately 7 to 14 kg at study initiation. Dogs will be assigned to dose groups such that the heaviest males and females are used together.

3. Care and Housing:

General procedures for animal care and housing will be in accordance with DHHS Publication No. (NIH) 86-23 (Revised, 1985) and the U.S. Department of Agriculture through the Animal Welfare Act (7 USC 2131), 1985 and Animal Welfare; Standards incorporated in 9 CFR Part 3, 1991. The dogs will be housed individually in stainless steel cages.

4. Diet and Water Supply:

A certified, commercial, dry chow or meal with the following minimum

composition will be used.

- Approximately 10% moisture
- At least 20% crude protein
- Approximately 5% fat
- Nutritionally adequate amounts of minerals
- Both water soluble and fat soluble vitamins

Dogs will have exposure to their daily ration for a total period of 1 to 2 hours per day. The quantity of the daily ration will be sufficient to meet nutritional requirements. The water source will be the public supply given ad libitum. No contaminants will be present in the feed or water which could interfere and affect the results of the study.

5. Quarantine:

Dogs will be held in quarantine for a minimum of 14 days prior to baseline measurements. A complete physical examination including a fecal examination for internal parasites, clinical pathology, body weight and rectal temperature will be performed on dogs within 7 days of delivery. See Section II.C.4.d. for clinical pathology. All data will be recorded. If the physical examination indicates the presence of internal parasites, dogs will be administered a vermifuge approved by the NCI Project Officer. Only positive dogs will be treated for parasites. If treatment is necessary, a minimum of 28 days will elapse prior to initial dosing. The dogs selected for study will be in good physical condition.

6. Animal Identification:

Dogs will be uniquely identified by ear tattoo number or letter combination. Positive identification will be required at least after every cage change, blood sampling and dosing.

C. Experimental Design

1. Group Assignments:

Two dogs of each sex will be randomly assigned to one of three different dose groups plus a vehicle control group, as shown.

	DOSE	# of Dogs	# of Dogs Sacrificed[a]

GROUP	(mg/kg/dose)	Study Start[a]	DAY 29	DAY 57
I (VCTL)	0.0	4	2	2
II	TBD	4	2	2
III	TBD	4	2	2
IV	TBD	4	2	2

[a] Equal numbers of males and females.

2. Route of Administration and Reason for Choice:

The test article will be given orally because this is an intended route of administration of this compound in humans.

3. Dosing Procedure:

The test article will be administered to Groups II, III, and IV orally by gavage every 8 hours for 28 consecutive days for a total of 84 doses. Dogs in the vehicle control group (Group I) will receive a volume of vehicle equivalent to the greatest volume administered on a mL/kg basis every 8 hours for 28 consecutive days for a total of 84 doses. Dose calculations for amount of drug given to each dog will be checked by a second individual who will initial and date the verification.

4. Measurements:

a. Clinical Signs:

PRETEST - Observe dogs daily and record any abnormal clinical signs. Baseline body temperature will be taken once on day -10 and once on day -3.

TEST - Adverse clinical signs will be observed and recorded at least twice daily during the dosing period and at least once daily thereafter or more often as clinical signs warrant. Body temperatures will be measured and recorded 3 hours after dosing on day 1 and twice a week during the dosing period, on

study day 29 and weekly thereafter on the same day each week at approximately the same time each day, or more often as clinical signs warrant.

b. Body Weight:

PRETEST - Body weights will be recorded on days -10 and -3. Food intake will be quantitatively evaluated and water intake will be qualitatively evaluated on days -10 and -3.

TEST - Body weights will be recorded twice weekly during the dosing period, on study day 29 and weekly thereafter. Food intake will be quantitatively recorded twice weekly during the dosing period, on study day 29 and weekly thereafter. Water intake will be qualitatively evaluated twice weekly during the dosing period, on study day 29 and weekly thereafter.

c. Ophthalmology:

PRETEST - Ocular examinations will be performed once during the pretest period (days -10 to -3) before the dogs are placed on study.

TEST - Ocular examinations will be performed on all dogs prior to necropsy.

d. Clinical Pathology:

PRETEST - All dogs will be fasted overnight and bled for clinical pathology on days -10 and -3. These baseline samples will be taken after the quarantine period is completed. Dogs that have aberrant values will not be used.

TEST - All dogs will be fasted overnight and blood drawn for clinical pathology on study days 4, 8, 11, 15, 18, 22, 25, 29, and weekly thereafter. Blood drawn on dosing days will be taken prior to treatment. Ideally, weekly blood samples should be drawn at the same time on the same days each week. The procedures will be performed according to the laboratory's SOP but in no case will dogs be bled from the treatment site. A blood sample will be obtained prior to the necropsy of each dog sacrificed in a moribund condition.

PLASMA MIXING EXPERIMENT - On the first day that the prothrombin time for a particular dog exceeds the mean of the pretest values by two or more times, a second plasma aliquot should be mixed with an equal volume of plasma from a vehicle control dog and then remeasured for prothrombin time.

Hematology:

Erythrocyte count (RBC) - $10^6/mm^3$
Hemoglobin (HGB) - g/dL
Hematocrit (HCT) - %
Mean corpuscular volume (MCV) - fl
Mean corpuscular hemoglobin (MCH) - pg
Mean corpuscular hemoglobin concentration (MCHC) - g/dL
Platelet count (Plate) - $10^3/mm^3$
Reticulocyte count (RETIC) - % RBC
Total leukocyte count (WBC) - $10^3/mm^3$
Differential leukocyte count - %
Nucleated red blood cell count (nRBC) - nRBC/100 WBC

Clinical Chemistry:

Blood urea nitrogen (BUN) - mg/dL
Serum aspartate aminotransferase (AST) - I.U./L
Serum alanine aminotransferase (ALT) - I.U./L
Alkaline phosphatase (Alk. Phos.) - I.U./L
Serum glucose (BS) - mg/dL
Prothrombin time (PT) - sec
Creatinine (CREAT) - mg/dL
Total protein (T PROTEIN) - g/dL
Sodium (Na) - meq/L
Potassium (K) - meq/L
Chloride (Cl) - meq/L

e. Plasma Drug Level Determination:

Blood samples will be drawn from two dogs (one sex pair) at 0 (immediately before dosing), 30, 60, 90 and 120 minutes, 3, 4, and 6 hours after the first dose on days 4, 11, 18, and 25. An aliquot of each sample (approximately 2 ml) will be mixed with EDTA. The samples will be centrifuged and the plasma frozen at least at -20^oC until analyzed. A blood sample will be drawn for plasma drug analysis prior to the sacrifice of moribund dogs

during the dosing period. Analytical procedures have been previously supplied in the compound information.

f. Necropsy Procedure:

UNSCHEDULED SACRIFICE - Moribund animals will be sacrificed to minimize the degree of postmortem autolysis. The authorization to sacrifice moribund dogs will be made by the Study Director or other qualified individual after examination of the dogs. The sacrifice of moribund dogs should follow the same procedure as a scheduled sacrifice when possible. If a dog is found dead outside of normal working hours, the dog will be necropsied as soon as possible with the carcass refrigerated (not frozen) in the interim period (not to exceed 24 hr). Body weight will be taken and tissues will not be discarded because of postmortem autolysis.

SCHEDULED SACRIFICE - One male and one female dog from each drug dose group will be sacrificed on day 29. The remaining animals will be sacrificed on study day 57 unless continuation is warranted (i.e., if there is no reversal of toxicity). All dogs will be killed by exsanguination after administration of a barbiturate overdose. The most severely affected dogs in each dose group will be sacrificed on study day 29.

The dogs will be fasted overnight prior to necropsy. Body weights and clinical pathology samples will be taken on the day of necropsy.

A complete necropsy and all antemortem observations will be recorded for each dog and commented on or confirmed at necropsy. Clinically normal dogs should also be so indicated. A pathologist will be available to examine any unusual findings.

The tissues listed below will be examined, sampled and fixed in cold, buffered neutral 10% formalin.

* Adrenal glands (2)
Aorta
* Bone, femoral head with articular surface
* Bone marrow, sternum
Bone marrow-rib, costochondral junction
* Brain

* Cecum
* Colon
* Duodenum
* Epididymides (2)
* Esophagus
* Eyes (2)
* Gall bladder
* Gonads (2)
* Gross lesions
* Heart
* Ileum
* Jejunum
* Kidneys (2)
* Lip
* Liver
* Lungs
* Lymph nodes (bronchial, mandibular, mesenteric)
* Mammary gland (when present in regular abdominal skin section)
* Pancreas
* Parathyroid gland (when present in regular thyroid gland section)
* Pituitary gland
* Prostate gland
* Salivary gland, mandibular
* Sciatic nerve
* Skeletal muscle
* Skin: 1. ventral abdomen 2. injection site
* Spinal cord, thoracolumbar (cervical and posterior lumbar spinal cord examined if nervous system signs present)
* Spleen
* Stomach (cardiac, fundic, and pyloric)
* Thymus
* Thyroid glands
  Tongue
* Tonsils (2)
* Trachea
* Urinary Bladder
* Uterus

The identification mark from the dog will be preserved in fixative.

A sample of all fixed tissues marked with an asterisk (*) will be embedded and put into blocks.

g. Microscopic Pathology:

Sections of the above tissues marked with an asterisk (*) will be cut approximately 5 microns thick and stained with hematoxylin and eosin.

All tissues will be examined microscopically by a pathologist. Records of gross findings for a specimen from postmortem observations shall be available to the pathologist when examining that specimen histopathologically.

All lesions will be categorized either as drug-related or nondrug related. Pathology code tables found at the National Toxicology Program website http://ntp.niehs.nih.gov/?objectid=72016020-BDB7-CEBA-F3E5A7965617C1C1, should be used.

Reporting of Pathology data should follow as closely as possible guidelines presented in “Toxicologic Pathology, 34:806–809, 2006”

III. QUALITY ASSURANCE

A. Type of Study

This is a nonclinical laboratory study and will require compliance with the FDA Good Laboratory Practice Regulations. Data from this study will be included as part of a final report to be submitted to the FDA.

B. Standard Operating Procedures

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the standard operating procedures of the laboratory and any deviations will be documented.

C. Protocol Amendments

All changes in or revisions of an approved protocol and the reasons therefore will be documented, signed, and dated by the Principal Investigator, Study Director and the NCI Project Officer. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the NCI Project Officer, but a written

amendment will follow.

D. Records

Data will be verified by the laboratory's Quality Assurance Unit and stored in accordance with the Good Laboratory Practice Regulations.

IV. REPORTING AND DISCUSSION OF DATA

A. Progress Reports

Status reports summarizing the progress of the study will be provided at monthly intervals. These reports will detail the status of the study on the reporting date, any problems encountered and proposed means of resolution.

B. Final Report

The data and results of this study including microscopic pathology will be submitted as a separate draft report, due 30 working days after the last dog sacrifice in this study. The final report will be due 30 working days after return of the draft report for revision.

This report will accurately and completely describe the study design, procedures and findings, present an analysis and summary of the data followed by the conclusions derived from the analyses. The report will also include: (a) a cover page which will include the title, contract number, authors, laboratory address, dates of initiation and completion, and sponsor; (b) the NTIS Report Documentation Page, to be placed at the beginning of the final report; (c) a comprehensive summary to be placed after the NTIS page; and (d) the signature of the Study Director and any others deemed necessary; (e) a table of contents; and (f) a statement prepared and signed by the Quality Assurance Unit which will refer to all phases of the study and where the raw data records, reports and samples are stored.

Protocol Approvals:

Study Director: _____ _____
(Date)

Principal Investigator: _____ _____
(Date)

Project Officer: _____ _____
(Date)